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A Novel Tertiary Pyridostigmine Derivative [3-(*N,N*-Dimethylcarbamoyloxy)-1-Methyl- Δ^3 -Tetrahydropyridine]: Anticholinesterase Properties and Efficacy against Soman¹

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A Novel Tertiary Pyridostigmine Derivative [3-(*N,N*-Dimethylcarbamoyloxy)-1-Methyl- Δ^3 -Tetrahydropyridine]: Anticholinesterase Properties and Efficacy against Soman. RAY, R., CLARK, O. E., III, FORD, K. W., KNIGHT, K. R., HARRIS, L. W., AND BROOMFIELD, C. A. (1991). *Fundam. Appl. Toxicol.* 16, 267-274. In an effort to develop an effective centrally acting pretreatment compound against organophosphorus poisons, the tertiary pyridostigmine (Pyr) derivative 3-(*N,N*-dimethylcarbamoyloxy)-1-methyl- Δ^3 -tetrahydropyridine (THP) was synthesized and studied for its anticholinesterase properties, as well as its efficacy against soman intoxication in guinea pigs. Injection of THP (262 μ g/kg, im) into adult male guinea pigs caused inhibition of blood (30%) and brain (25%) acetylcholinesterase (AChE), showing that THP penetrates the blood-brain barrier. Pyr (131 μ g/kg, im) caused AChE inhibition in the blood (59%), but not in the brain. The inhibitory potencies of THP and Pyr were compared by determining their IC₅₀ values for *in vitro* inhibition of both AChE (brain, erythrocyte) and pseudo-cholinesterase (plasma) in three mammalian species (guinea pig, rat, rabbit). THP, although effective in inhibiting both types of cholinesterase, was in general less potent than Pyr. Pretreatment of guinea pigs with THP (262 μ g/kg, im) plus Pyr (131 μ g/kg, im), 30 min prior to subcutaneous soman challenge, with no antimuscarinic or oxime treatment, protected 60% of the animals against 2 \times LD₅₀ of soman. Neither THP nor Pyr alone was effective. The protective pretreatment regimen did not prevent convulsions, but shortened the recovery time in surviving animals (median recovery time 1.6 hr, compared to 24 hr in control and other groups of animals pretreated with THP or Pyr alone). A combination of THP and Pyr thus appears to provide a means of evaluating the relative importance of selective peripheral plus central vs peripheral AChE protection against soman. © 1991 Society of Toxicology.

The toxicity of organophosphorus compounds (OPs) in animals is believed to be due to their inhibition of acetylcholinesterase (AChE) at synaptic junctions (Taylor, 1985). Because of

their highly lipophilic nature, OPs can readily penetrate into the central nervous system (CNS) to cause central toxicity in addition to peripheral toxicity. Reversible blockade of AChE in both central and peripheral compartments may therefore be required for protection against OPs. The addition of pyridostigmine (Pyr) pretreatment to the standard therapy of atropine and 2-PAM (*N*-methylpyridinium-2-aldoxime chloride) for OP poisoning enhances the protective efficacy (Lennox *et al.*, 1985). Carbamates like Pyr protect

¹ In conducting the research described in this report, the investigators adhered to the *Guide for the Care and Use of Laboratory Animals*, NIH Publication No. 85-23, revised 1985.

² The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Army or the Department of Defense.

AChE by maintaining a reactivatable pool of carbamylated AChE that can be spared from inhibition during exposure to OPs. However, being a quaternary compound, Pyr protects only peripheral AChE. To circumvent this problem, we synthesized the tertiary Pyr derivative 3-(*N,N*-dimethylcarbamoyloxy)-1-methyl- Δ^3 -tetrahydropyridine (THP) and studied its anticholinesterase (anti-ChE) properties as well as its efficacy against soman in guinea pigs. We proposed earlier that this novel tertiary Pyr derivative THP (Fig. 1) should protect central AChE, and therefore protect against the irreversible anti-ChE agent soman (Ray and Clark, 1987; Ray *et al.*, 1989). The results of these studies are presented in this report.

MATERIALS AND METHODS

Animals. Hartley guinea pigs (300–350 g), Sprague-Dawley rats (200–250 g), and New Zealand rabbits (2.5–3.0 kg), all males, were obtained from Charles River Laboratories (Wilmington, MA). The animals were housed in a temperature- and humidity-controlled atmosphere with 12-hr periods of light and dark and were provided food and water *ad libitum*. During experimentation (handling, injection, observation), the animals were housed individually without food and water.

Chemicals. Pyridostigmine bromide (>98.5% pure) and tetrahydropyridostigmine hydrobromide (>99.5% pure) were obtained from the Walter Reed Army Institute of Research, Washington, D.C., the latter being custom synthesized by a chemical company according to the specifications provided. Acetylthiocholine iodide, 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB), BW284C51, and isomPA were purchased from Sigma (St. Louis, MO). Acetyl-1- β -[14 C]methylcholine bromide was obtained from Amersham (Arlington Heights, IL). Soman (pinacolyl

methylphosphonofluoridate) was obtained from the Chemical Research Development and Engineering Center (Aberdeen Proving Ground, MD) and subsequently analyzed (>99% pure) and supplied to us by the Analytical Chemistry Branch of this Institute. Buffers and other chemicals used were analytical grade.

Preparation of brain homogenate. Animals (guinea pigs, rats, rabbits) were anesthetized by carbon dioxide inhalation and decapitated. Whole brains were removed, freed of adhering blood, washed in ice-cold isotonic saline, minced with a fine pair of scissors, and homogenized in 4 vol of ice-cold buffered 0.32 M sucrose solution to prepare a 20% (wet wt/vol) homogenate. Aliquots (0.5 ml) were stored frozen at -20°C until use.

Preparation of blood plasma. Ten-milliliter samples of heparinized whole blood from each animal species were centrifuged (1200g) at room temperature for 10 min. Aliquots (0.5 ml) of supernatants (plasma) were stored frozen at -20°C until use.

Preparation of red blood cell (RBC) ghosts. After plasma was removed from 10-ml whole blood aliquots (see above), the remaining red blood cells were suspended in 10 ml ice-cold isotonic saline and then centrifuged. Suspension and centrifugation were repeated once more. Washed RBCs were resuspended in 10 ml hypotonic 10 mM Tris-HCl buffer, pH 7.2, and were left on ice for 60 min. The suspension was spun at 16,300g for 60 min at $2-4^{\circ}\text{C}$. The supernatant was removed, and the RBCs were again washed in Tris-HCl buffer, left on ice for 60 min, and centrifuged. This step was repeated until the sediment was almost white. Final washed sediments were dispersed in 5 ml of 50 mM sodium phosphate buffer, pH 7.2, containing 0.5 M NaCl, 1% Triton X-100, and 100 μM disodium EDTA. The suspension was vortexed vigorously to extract ChE from the membrane into the solution and then centrifuged at 16,300g for 30 min at $2-4^{\circ}\text{C}$. The supernatant was collected and 0.5-ml aliquots were stored at -20°C until use.

Assay of ChE. Cholinesterase assays were performed by either of the two methods. In the colorimetric automated microassay (Ray and Clark, 1988a), based on the method of Ellman *et al.* (1961) as described by Brogdon and Dickinson (1983), acetylthiocholine (ATCH) iodide was used as the substrate, and DTNB was used as the color reagent.



FIG. 1. Structures of test compounds.

In the radiometric method according to a modification (aqueous perchloric acid quencher, Talbot *et al.*, personal communication) of the method of Siakotos *et al.* (1969), acetyl- β -[^{14}C]methylcholine bromide was used as the AChE-specific substrate.

The reason for using the two different ChE assays follows. The colorimetric assay was used to compare the potencies of the test compounds for inhibiting ChE (AChE or pseudo-(butyryl)ChE) in different tissues. Moreover, the automated microassay was very suitable for conducting the large number of assays for testing the two compounds and nine tissues in an efficient and dependable (with many replicates) manner. Besides testing whether THP penetrated the blood-brain barrier as described below, the radiometric assay was also used to measure specifically AChE inhibition in brain and blood due to doses of THP (262 $\mu\text{g/kg}$) and Pyr (131 $\mu\text{g/kg}$) that protected animals against soman. The use of the colorimetric assay in this experiment would require an additional pseudo-ChE inhibitor and would complicate the interpretation of results. A comparison of the colorimetric and the radiometric methods by measuring the time course of a purified electric eel AChE activity produced identical results (data not shown). We found by using the colorimetric method the IC_{50} of the specific AChE inhibitor compound BW284C51 for inhibiting rat brain AChE to be $3 \times 10^{-8} \text{ M}$, which was similar ($5 \times 10^{-8} \text{ M}$) to the value reported by Siakotos *et al.* (1969) using the radiometric method.

Determination of AChE inhibition in guinea pig blood and brain in vivo by THP. Experimental groups of four guinea pigs were injected im (hind leg) with normal saline alone (control), THP, or Pyr in saline. In these groups, one animal was used for each time point. Blood samples (50 μl) were drawn from each animal by clipping the tip of the toenail just before the injection (zero time) and 15, 30, and 60 min after injection. The animals were immediately euthanized after each time point and their brains removed, washed, and homogenized (20%, wet wt/vol) in 50 mM sodium phosphate buffer (pH 7.4). For the zero-time control animal, blood was drawn, saline was injected, and brain was removed as quickly as possible. AChE was assayed radiometrically as described above.

Determination of the efficacy of THP. Efficacy of THP against soman intoxication was tested in guinea pigs according to the method described by Harris *et al.* (1988). Briefly, one control and three experimental groups of animals were studied. In each group of 10 guinea pigs, animals were randomly assigned to individual cages. The three separate groups of experimental animals were pretreated by injecting im with (a) Pyr (131 $\mu\text{g/kg}$) alone, (b) THP (262 $\mu\text{g/kg}$) alone, and (c) Pyr (131 $\mu\text{g/kg}$) plus THP (262 $\mu\text{g/kg}$) 30 min prior to a subcutaneous (sc) challenge with $2 \times \text{LD}_{50}$ (60 $\mu\text{g/kg}$) of soman. The dosage of Pyr was calculated based on the equation relating dosage of carbamate to inhibition of whole blood AChE in guinea pigs for approximately 70% inhibition, according to Lennox

et al. (1985). This level of ChE inhibition has been demonstrated to offer protection against soman. The dosage of THP was selected to produce approximately 30% AChE inhibition in the brain. Control animals were pretreated with diluent (isotonic saline). All injections of pretreatment drugs were done in a volume equivalent to 0.5 ml/kg of body weight and of soman in a volume equivalent to 1.0 ml/kg. Following soman challenge, each guinea pig was monitored by two observers. For the first 15 min, animals were continuously observed for convulsions. They were graded for visible signs of intoxication at less than 0.25, 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, and 24 hr. Also, 24-hr mortality was recorded. Severity scores and signs of intoxication were graded as follows. Severity was scored for each animal—Mean (for the pair of observers monitoring the animal) of the sum of points for signs at the following times after soman: 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, and 24 hr + 5 points if convulsions occur (zero, otherwise) before 0.25 hr. Convulsions = 5 points; prostration = 4 points; tremors/or fasciculations = 3 points compulsive chewing/or salivation, = 2 points; and increased locomotion = 1 point. Maximum severity score = 50, or 10 observation times \times 5 points/observation time. If death occurred during the first 3 hours, the death time was noted to the nearest observation time. In the same manner, a recovery time was noted for each survivor from observation until 5 hr and at 24 hr.

Data analysis. Data in Table 3 were analyzed by the Fisher's exact test (number of dead and percentage convulsions) and the Kruskal-Wallis test (time to death and severity score). Data in Fig. 2, 3, and 4 were analyzed by the Mann-Whitney *U* test at each concentration.

RESULTS

ChE Inhibitory Potency of THP

The anti-ChE potency of THP was determined colorimetrically for brain and blood (plasma, RBC ghosts) *in vitro* from different mammalian species. High salt (0.5 M NaCl) and detergent (1% Triton X-100) were present in all tissue samples (brain homogenate, plasma, RBC ghosts) to fully activate ChE. The synthesis of THP-HBr was achieved by chemical reduction of pyridostigmine bromide using sodium borohydride (NaBH_4) and hydrogen bromide (HBr) following the method of Horstmann and Haefelinger (1985). The anti-ChE potency of THP was therefore compared with that of Pyr for all tissues tested from the different animal species, guinea pig (Fig. 2), rat (Fig. 3), and rabbit (Fig. 4). The relative

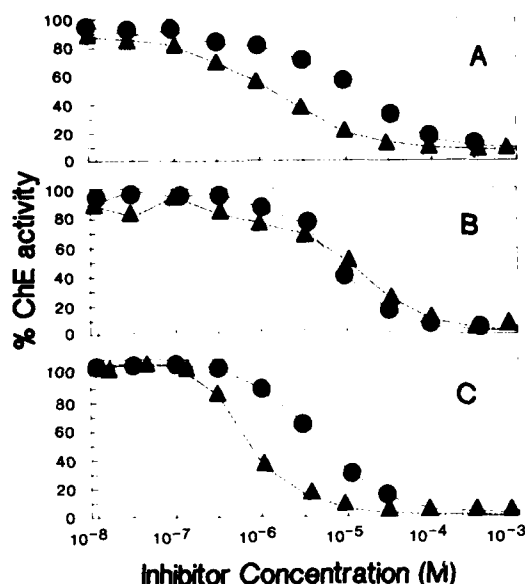


FIG. 2. Inhibition of guinea pig brain (A), plasma (B), and RBC ghost (C) ChE by THP (●) and Pyr (▲). Enzyme assays were done colorimetrically by incubating different tissues at varying inhibitor concentrations for an optimal level of activity. Results were expressed as percentage of control (100% activity without inhibitor). Control ChE activities were 28, 112, and 8.33 nmol substrate hydrolyzed/mg protein/min for brain, plasma, and RBC ghosts, respectively. Data points are means \pm SE of six replicate assays. No SE bars are shown because the values are smaller than the symbols. THP was significantly ($p < 0.05$) different from Pyr at 10^{-7} to 3×10^{-5} M (A and C).

potencies (IC_{50}) of THP and Pyr, derived from the enzyme inhibition versus inhibitor concentration curves shown in Figs. 2, 3, and 4, are summarized in Table 1. It is seen that both THP and Pyr effectively inhibit ChE in the different tissues tested. However, with the exception of guinea pig plasma, the concentration-response curve of THP is always to the right of that of Pyr, indicating that THP is a poorer ChE inhibitor.

Permeability of Blood-Brain Barrier to THP

In order to test whether the reduced Pyr derivative THP would penetrate through the

blood-brain barrier, inhibition of blood and brain AChE following im (hind leg) injection of THP into guinea pigs was measured as described under Materials and Methods. The inhibition of blood and brain ChE due to im injection of Pyr into guinea pigs was also measured for comparison. The dosage of Pyr was 131 μ g/kg, which was based on the equation relating dosage of carbamate to inhibition of whole blood AChE in guinea pigs for approximately 70% inhibition according to Lennox *et al.* (1985). Since THP is a poorer inhibitor than Pyr (Figs. 2, 3, 4 and Table 1), the dosage of THP was arbitrarily twice (262 μ g/kg) as much as Pyr. The results presented in Table 2 show that at 262 μ g/kg dose THP inhibited AChE approximately 30% in both blood and brain in 15 min. This level of inhibition remained until 60 min. Pyridostigmine, in similar experiments, caused $58.50 \pm 10.65\%$

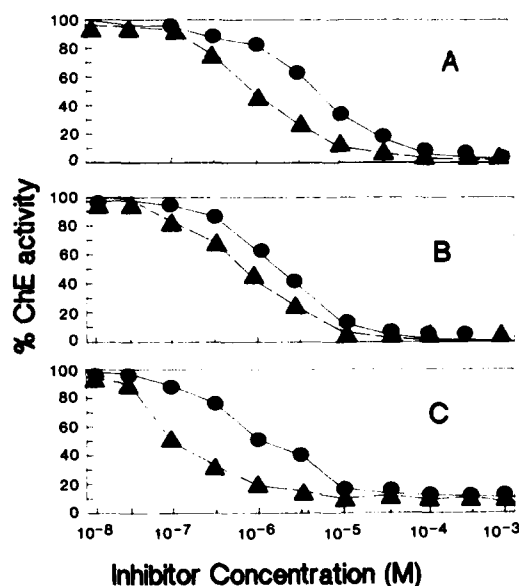


FIG. 3. Inhibition of rat brain (A), plasma (B), and RBC ghost (C) ChE by THP (●) and Pyr (▲). Control ChE activities were 45.67, 62.67, and 0.33 nmol substrate hydrolyzed/mg protein/min for brain, plasma, and RBC ghosts, respectively. THP was significantly ($p < 0.05$) different from Pyr at 3×10^{-7} to 3×10^{-5} M (A) and at 10^{-7} to 10^{-5} M (B and C). Other details are as in Fig. 2.

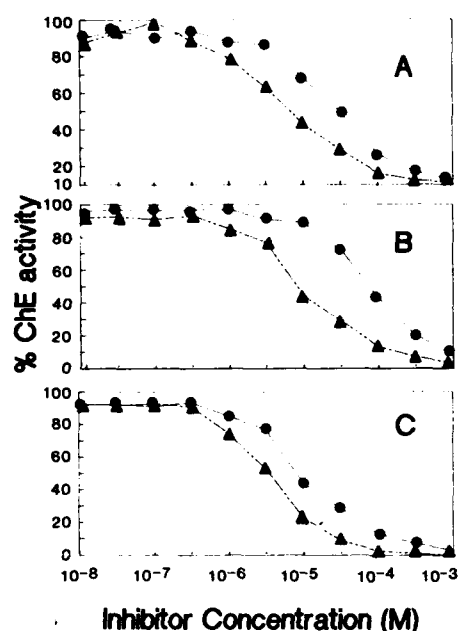


FIG. 4. Inhibition of rabbit brain (A), plasma (B), and RBC ghost (C) ChE by THP (●) and Pyr (▲). Control ChE activities were 9.33, 55.67, and 6.67 nmol substrate hydrolyzed/mg protein/min for brain, plasma, and RBC ghosts, respectively. THP was significantly ($p < 0.05$) different from Pyr at 10^{-6} to 10^{-4} M (A), at 10^{-6} to 10^{-3} M (B), and at 10^{-6} to 3×10^{-4} M (C). Other details are as in Fig. 2.

AChE inhibition in the blood and no inhibition in the brain. These results show that THP penetrates through the blood-brain barrier and causes AChE inhibition.

Efficacy of THP Against Soman

Protection by THP against soman poisoning was tested in guinea pigs as described under Materials and Methods. The results shown in Table 3 indicate that im pretreatment with 262 $\mu\text{g/kg}$ THP plus 131 $\mu\text{g/kg}$ Pyr (brain AChE inhibition, $\sim 30\%$; blood AChE inhibition, $> 70\%$), 30 min prior to sc soman challenge, and with no antimuscarinic or oxime treatment, protects 60% of the animals against $2 \times \text{LD}_{50}$ of soman and prolongs time to death.

TABLE 1

INHIBITION OF BRAIN, PLASMA, AND RBC GHOST ChE BY THP AND Pyr IN DIFFERENT MAMMALIAN SPECIES

| Test compound | Brain | Plasma | RBC ghost |
|--|-------|--------|-----------|
| Guinea pig (IC_{50} , μM)* | | | |
| THP | 13.00 | 7.00 | 6.00 |
| Pyr | 1.50 | 12.00 | 0.80 |
| Rat (IC_{50} , μM)* | | | |
| THP | 5.50 | 2.00 | 3.50 |
| Pyr | 0.95 | 0.80 | 0.20 |
| Rabbit (IC_{50} , μM)* | | | |
| THP | 30.00 | 80.00 | 9.00 |
| Pyr | 6.00 | 8.00 | 3.10 |

* Inhibitory potency (IC_{50}) values, i.e., inhibitor concentrations (μM) needed to inhibit 50% of control enzyme activity *in vitro*, were obtained from enzyme inhibition-inhibitor concentration curves shown in Fig. 2, 3, and 4. THP was significantly ($p < 0.05$) different from Pyr for all tissues tested except guinea pig plasma.

Both results are highly significant. Inhibition of AChE in the blood and in the brain by THP (262 $\mu\text{g/kg}$) alone or in the blood by Pyr (131 $\mu\text{g/kg}$) alone provides no protection. Although pretreatment with THP + Pyr does not pre-

TABLE 2

INHIBITION OF BLOOD AND BRAIN AChE BY THP (262 $\mu\text{g/kg}$, im) IN GUINEA PIG *IN VIVO*

| Tissue | % AChE inhibition | | |
|--------|-------------------|------------------|------------------|
| | 15 min | 30 min | 60 min |
| Blood | 30.00 ± 5.01 | 29.33 ± 3.41 | 26.00 ± 6.82 |
| Brain | 25.33 ± 5.80 | 21.00 ± 6.14 | 26.00 ± 2.73 |

Note. The time course of AChE inhibition in blood and brain following THP injection was determined as described under Materials and Methods. Values are means \pm SEM of results from three separate experiments. Pyr (131 $\mu\text{g/kg}$, im) caused $58.50 \pm 10.65\%$ AChE inhibition in blood in 15 min, which remained at this level until 60 min, but it caused no AChE inhibition in brain.

TABLE 3
EFFECTS OF THP PRETREATMENT AGAINST SOMAN-INDUCED LETHALITY
AND PHYSICAL INCAPACITATION IN GUINEA PIGS

| Pretreatment ^a | No. | Mortality | C/sub-C effects ^b | | |
|--------------------------------------|-------|--|------------------------------|--|--|
| | | Time to death (hr) Md (extremes) | %C | Severity score ^c (0-50) Md (extremes) | Recovery time (hr) Md (extremes) |
| Pyr (131 µg/kg) | 9/10 | 0.5 (0.25; 24) | 80 | 15 (6; 33) | 24 |
| THP (262 µg/kg) | 8/10 | 0.5 (0.25; 24) | 90 | 15 (11; 35.5) | 24 (4; 24) |
| Pyr (131 µg/kg) + THP (262 µg/kg) | 4/10* | 2.5** (1; 24) | 70 | 20.5 (7; 32.5) | 1.625 (0.5; 24) |
| Vehicle (saline) | 9/10 | 0.5 (0.25; 0.5) | 70 | 13 (6; 25) | 24 |

^a All pretreatments were injected im into hind leg muscles 30 min prior to soman (60 µg/kg, sc; 2 × LD50).

^b C/Sub-C effects, convulsive/subconvulsive effects.

^c Severity score for each animal was obtained as described under Materials and Methods.

* Pyr + THP was significantly ($p < 0.03$) different from Pyr and vehicle.

** Pyr + THP was significantly different from THP ($p < 0.03$) and vehicle ($p < 0.006$).

vent convulsions, it markedly shortens the recovery time (median recovery time of 1.6 hr, compared with 24 hr in control and other groups). Statistical significance of this recovery time could not be obtained due to the low numbers of survivors in groups other than THP + Pyr.

DISCUSSION

As mentioned above, the major toxicity of OPs is thought to be due to the inhibition of AChE at cholinergic synapses leading to an excessive accumulation of acetylcholine and overstimulation of acetylcholine receptors. Therefore, the conventional therapy of OP poisoning is the use of an antimuscarinic agent such as atropine to protect muscarinic receptors and an oxime such as 2-PAM to reactivate inhibited AChE. Although effective against OPs like sarin, tabun, and VX, this therapy regimen is not very useful against soman because soman-inhibited AChE ages rapidly and reactivates poorly (Lennox *et al.*, 1985). For

this reason, the use of pretreatment compounds like Pyr that may protect AChE from inhibition by OPs may be beneficial against soman. The need for protection of AChE in both the CNS and the periphery has been discussed above. The quaternary carbamate Pyr which does not permeate through the blood-brain barrier is currently used for protection of AChE in the periphery against OP poisoning due to high efficacy and low toxicity. Other researchers have investigated the ability of the tertiary carbamate physostigmine (Phy) to protect AChE in the CNS (Harris *et al.*, 1988). However, Phy has been found to produce both behavioral and cellular toxicity in the CNS (Harris *et al.*, 1984).

In the current study, we investigated the tertiary Pyr derivative THP as a pretreatment compound against soman poisoning. The initial idea was to test the efficacy of a combination of two carbamates, Pyr (for peripheral protection) and THP (for central protection) against soman intoxication. The combination of the quaternary compound Pyr and its ter-

tiary derivative THP would provide the following advantages: (a) both peripherally and centrally acting pretreatment compounds would be of the same chemical class, and (b) it would allow selective levels of peripheral and central AChE inhibition, so that an unnecessarily excessive central AChE inhibition from a large dose of a tertiary carbamate to achieve a high level of peripheral protection would be avoided.

To provide protection against OPs, both pretreatment compounds should be able to effectively inhibit AChE. The anti-ChE properties of Pyr are well characterized. The test compound is new (Fig. 1). It has also been shown that the anti-ChE potency of carbamates, e.g., Phy and Pyr, for inhibiting purified electric eel AChE is altered by chemical modification of these compounds (Brossi *et al.*, 1986; Ray and Clark, 1988b). Moreover, the properties of ChEs are different in different tissues in different animal species (Silver, 1974). It was therefore worthwhile to characterize the anti-ChE properties of the new compound THP. As can be seen from our results (Figs. 2, 3, 4, and Table 1) THP, although somewhat less potent than Pyr, can effectively inhibit ChEs, and therefore may have potential as a pretreatment compound. We characterized the nature of ChE (AChE or pseudo-ChE) in different tissues (brain homogenate, plasma, and RBC ghosts of guinea pig, rat, and rabbit) listed in Table 1 by using selective inhibitors, BW284C51 (AChE inhibitor) and iso-OMPA (pseudo-ChE inhibitor). In all animal species, brain and RBC ghosts were predominantly AChE, whereas plasma was predominantly pseudo-ChE (data not shown). The results shown in Table 1 indicate that neither THP nor Pyr has any remarkable difference in their potency in inhibiting AChE (brain, RBC ghosts) compared to pseudo-ChE (plasma) in any animal species.

The test compound THP, obtained by chemical reduction of Pyr to yield a tertiary derivative, appears to cross the blood-brain barrier and inhibit brain AChE (Table 2). It is

therefore expected that THP should function as a centrally acting pretreatment compound. The dose of THP (262 $\mu\text{g/kg}$, im) alone or in combination with Pyr (131 $\mu\text{g/kg}$) used in the efficacy experiments (Table 3) did not produce any observable behavioral abnormality (physical responses, e.g., tremor, convulsion, salivation, chewing) in the animals during the 30-min period preceding their exposure to soman. However, this combination of THP plus Pyr provided significant protection to these animals against a moderate challenge ($2 \times \text{LD}_{50}$) of soman without any antimuscarinic or oxime therapy. Neither THP nor Pyr alone provided any protection against this soman challenge. The results of our study thus suggest that THP is a potential centrally acting pretreatment compound, which also provides a means of evaluating the relative importance of selective peripheral plus central versus peripheral AChE protection against OPs. Pyr does not penetrate through the blood-brain barrier, and therefore does not inhibit central ChE. It is therefore possible to protect ChE in the periphery alone by using Pyr, or in both peripheral and central nervous systems by using a mixture of Pyr and THP. The relative proportions of Pyr and THP in the mixture can also be varied to achieve any desired levels of ChE inhibition in either of the central or peripheral compartments, particularly to avoid the adverse effects of an excessive central ChE inhibition.

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